

A change of phenolic acids content in poplar leaves induced by methyl salicylate and methyl jasmonate

AN Yu¹, SHEN Ying-bai^{1*}, WU Li-juan², ZHANG Zhi-xiang¹

¹ College of Biological Science and Biotechnology, Beijing Forestry University, Beijing 100083, P. R. China

² Department of Science and Technology, Beijing Forestry University, Beijing 100083, P. R. China

Abstract: The contents of seven different phenolic acids such as gallic acid, catechinic acid, pyrocatechol, caffeic acid, coumaric acid, ferulic acid and benzoic acid in the poplar leaves (*Populus Simonii* × *Populus Pyramibalis* c.v and *Populus deltoids*) suffocated by Methyl jasmonate (MeJA) and Methyl salicylate (MeSA) were monitored for analyzing their functions in interplant communications by using high-pressure liquid chromatography (HPLC). The results showed that the contents of phenolic acids had obviously difference in leaves exposed to either MeSA or MeJA. When *P. deltoides* leaves exposed to MeJA or MeSA, the level of gallic acid, coumaric acid, caffeic acid, ferulic acid and benzoic acid was increased, gallic acid in leaves treated with MeJA comes to a peak at 24 h while to a peak at 12-d having leaves treated with MeSA. When *P. Simonii* × *P. Pyramibalis* c.v leaves were exposed to MeJA or MeSA, the level of gallic acid, pyrocatechol and ferulic acid was increased; The catechinic acid and benzoic acid had a little drop; The caffeic acid and coumaric acid were undetected in both suffocated and control leaves. This changed pattern indicated that MeJA and MeSA can act as airborne signals to induce defense response of plants.

Keywords: Methyl salicylate (MeSA); Methyl jasmonate (MeJA); Airborne signal; Phenolic acid; Induce resistance

CLC number: Q946.8

Document code: A

Article ID: 1007-662X(2006)02-0107-04

Introduction

Naturally, plants often encounter various challenges simultaneously from biotic and abiotic stresses. Each environmental factor can activate different signalling pathways to response as a spatial and temporal defense (Dempsey *et al.* 1999; Ryan 2000). For protecting themselves from pathogen, mechanical or herbivore attack, plants release a complex blend of volatiles that provide valuable cues for herbivores and their natural enemies (Dicke 1999; Paré *et al.* 1999). These biochemical and physiological responses of plants often result in a tolerance or protection from further environmental challenges.

Several studies have shown that plants exposed to volatile compounds may increase the production of defensive chemicals even though they themselves have not been damaged (Dicke *et al.* 2001). Methyl jasmonate (MeJA), a volatile product of the octadecanoid signaling pathway, has been shown to induce the synthesis of defensive proteinase inhibitor in the treated plants and in neighboring plants (Farmer *et al.* 1990). Methyl salicylate (MeSA) is synthesized from salicylic acid (SA), which is an important component of systemic-acquired resistance (SAR) in several plants species (Shah *et al.* 1999). SAR is a pathogen-inducible resistance in tissue distant from the primary infection. Previous experiments showed that MeJA could mediate interplant communications in some plants (Farmer *et al.* 1990; McConn *et al.* 1997; Farmer *et al.* 1998). However, these experiments were on the herbaceous plants and there was limit evidence to show such a process in woody plant. Other

plant-derived volatile and MeSA have recently been reported as airborne signal in plant-plant communication (Shulave *et al.* 1997). We try to know whether MeJA and MeSA can travel through the atmosphere to activate plant defense and whether communication exists between neighboring plants within this experiment.

The resistance of plants towards insect and pathogen is based on cell wall lignification and phytoalexin synthesis (Wagner 1988). Phenolic acids play a key role in these reactions. Phenolic acids are a large class of plant secondary metabolite products distributing widely in the plant kingdom. The defensive role of phenolic acids relates to an increase in their content under stressed environment conditions, such as air pollution, UV radiation, infection or mechanical damage (Mieczyslaw 1999). Previous studies demonstrated that total phenols content increased in leaves when trees were damaged or exposed to volatile released by damaged plant (Baldwin *et al.* 1983), but there were few reports on the phenolic acids. Therefore, in this study, we used MeJA and MeSA to suffocate the poplars, then examined the contents of seven phenolic acids by high-pressure liquid chromatography (HPLC). Some of them are orthodihydroxy phenolic compounds and tannin which act as a strong inhibitor of exterior enzymes, others are phenylpropanoids which make up of plant cell wall, and the rest are key intermediates in biosynthesis pathway.

Materials and methods

Plant materials and treatment

Populus Simonii × *Populus Pyramibalis* c.v and *Populus deltoides* were cultivated in the pots under natural conditions in March. They were daily watered and irrigated by nutrition solution (5% Hoagland) every month. Trees were top-excised in April for more branches. The pots were moved frequently to avoid the roots growing outside, and the roots system remains intact.

Foundation item: This research is supported by National Natural Science Foundation of China (No.30170764)

Biography: AN Yu (1982-), female, postgraduate in College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, P. R. China. Email: anyu-02@163.com

Received date: 2005-09-29; **Accepted date:** 2005-12-25

Responsible editor: Zhu Hong

* Corresponding author: Email: ybshen@bjfu.edu.cn

The similar plants were chosen for minimizing the influence of difference between plants, and then they were exposed to MeSA or MeJA vapor inside an air-tight chamber. Airborne concentration of MeSA or MeJA in the chamber is about $0.8 \mu\text{mol}\cdot\text{L}^{-1}$ finally. The plants exposed to 100% of ethanol in the same condition were used as controls. The leaves were harvested with petiole after 4 h, 24 h, 6 d, 12 d and dried at 105°C for 4 h, then baked to be constant weight at 75°C in oven.

HPLC analysis

Chemicals

Gallic acid, catechinic acid, pyrocatechol, caffeic acid, coumaric acid, ferulic acid and benzoic acid were purchased from Sigma Chemical Company, and they all were chromatographic-grade reagents ($\geq 99\%$). Petroleum ether, methanol and phosphate were all analytical-grade reagent. Acetonitrile of chromatographic-grade reagent was obtained from Baker Company.

Extraction of phenolic acids

The dry leaves were ground to a fine powder in lender. Powdered material (3 g) was put into a flask, and 50-mL petroleum ether was added at room temperature with continuous stirring for 30 min and kept for overnight. The residual were shifted to flask and 50% methanol (50-mL) was added, refluxed for 1 h in a heat water bath and filtered. The filtrate was condensed and diluted to 25 mL with 50% methanol. 5-mL sample extract were loaded onto a Sep-pak C_{18} column that had been prewashed with 5-mL methanol and 5-mL distilled water, discard the initial eluent 1-mL, then collect 2-mL eluent for latter determination of phenolic acids.

Apparatus and conditions

The analysis of phenolic acids was performed by China Academy of Forestry Analytical Center. For HPLC analysis, the extracts were injected into *Water 244* HPLC equipped with UV detector ($254\text{nm}\times 0.1\text{AUFS}$) and *Kromasil C₁₈* ($0.4\text{cm}\times 25\text{cm}$). The mobile phase was composed of methanol-acetonitrile-water (35: 3: 62, v/v/v), and adjusted to pH 3.0 with phosphate. The flow rate was set to $0.7\text{mL}\cdot\text{min}^{-1}$. Injection volume was $10 \mu\text{L}$. Phenolic acids content was monitored and compared with that by the external standard method. Each treatment was repeated more than three times, and then the mean value was calculated.

Results

Effect of MeJA on phenolic acids change

Some studies reported that the plant secondary compounds would increase after being attacked by herbivores (Ryan 2000). In this study we also found that the changes at phenolic acid level also occurred in poplar leaves after MeJA treatment. When *populus deltoides* leaves were exposed to MeJA for 4 h, the content of all phenolic acids increased except for pyrocatechol, coumaric acid and benzoic acid (Fig. 1). Among phenolic acids, the content of gallic acid increased distinctly at 24 h, and then dropped off. Furthermore, during 6 days and 12 days, most phenolic acids remained at high level. It indicated that plant began to accumulate phenolics for about 4 h after the initial exposure to MeJA and continued to accumulate the phenolic acids for nearly 12 days.

For determining whether MeJA could activate phenolic acids synthesis in other plants, *P. simonii*×*P. pyramidalis* c.v were exposed to volatile MeJA for 24 h and the phenolic acids contents in their leaves were detected (Fig. 2). The results showed that the level of gallic acid and pyrocatechol in suffocated leaves were higher than those in control leaves; catechinic acid, ferulic acid and benzoic acid decreased a little; and caffeic acid and coumaric acid were undetected in both suffocated and control leaves.

Effect of MeSA on phenolic acids change

To understand how MeSA affect the contents of phenolic acids, we examined the contents of seven phenolic acids in *Populus deltoides* leaves exposed to MeSA for 4 h in the same container. The results showed that the level of gallic acid, catechinic acid, pyrocatechol and ferulic acid in suffocated leaves decreased (Fig. 3), and that ferulic acid had a significant decrease. At the same time, coumaric acid and caffeic acid increased. After 24 h, all phenolics increased except for catechinic acid; after six days, other phenolic acids increased diversely while gallic acid remained unchanged. Until 12 days, all phenolic acids increased, and gallic acid, catechinic acid and pyrocatechol were come to their maximum.

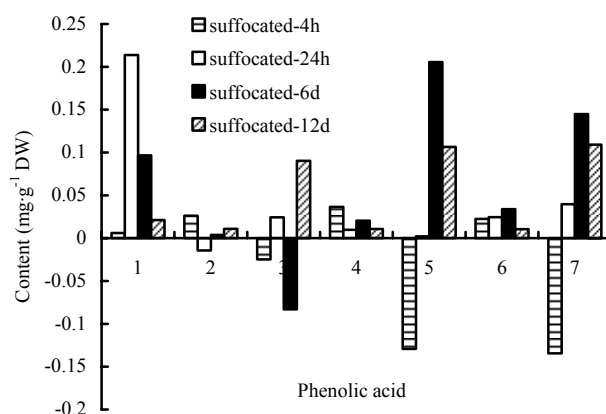


Fig. 1 Phenolic acids change in *Populus deltoides* leaves suffocated by MeJA.

The phenolic acids are net contents (except for the contents of phenolic acids in the control leaves). The number from 1 to 7 along the X-axis stands for gallic acid, catechinic acid, pyrocatechol, caffeic acid, coumaric acid, ferulic acid and benzoic acid, respectively.

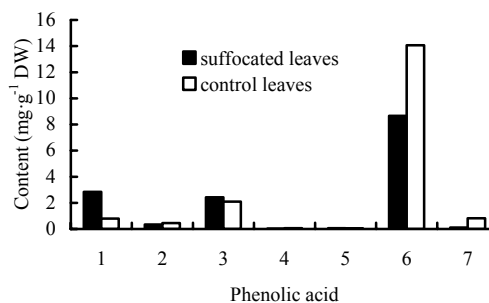


Fig. 2 Phenolic acids change in *P. simonii*×*P. pyramidalis* c.v leaves suffocated by MeJA.

The leaves treated with ethanol were used as control. The number from 1 to 7 along the X-axis stands for gallic acid, catechinic acid, pyrocatechol, caffeic acid, coumaric acid, ferulic acid and benzoic acid, respectively.

Meanwhile, the concentrations of phenolics were detected in *P. simonii* × *P. pyramidalis* c.v leaves after MeSA treatment. The level of gallic acid, pyrocatechol and ferulic acid in suffocated leaves was higher than that in control leaves. On the one hand, pyrocatechol had a significant increment (Fig. 4). On the other hand, catechinic acid and benzoic acid had a little drop. Caffeic acid and coumaric acid were undetected in both suffocated and control leaves. That is similar to the phenolic acids change induced by MeJA. Following the similar pattern, it suggested that MeSA may function as airborne signal induce the defense in woody plants

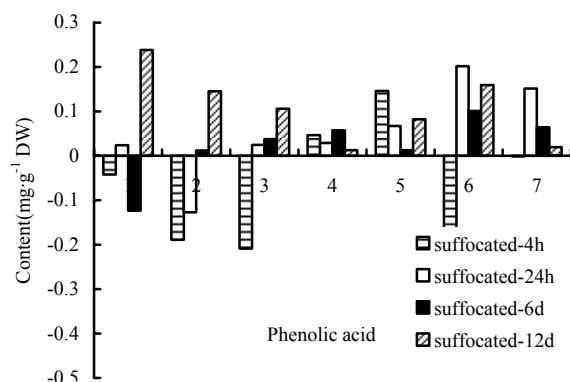


Fig. 3 Phenolic acids change in *Populus deltoides* leaves suffocated by MeSA.

The phenolic acids are net contents (except for the contents of phenolic acids in the control leaves). The number from 1 to 7 along the X-axis stands for gallic acid, catechinic acid, pyrocatechol, caffeic acid, coumaric acid, ferulic acid and benzoic acid, respectively.

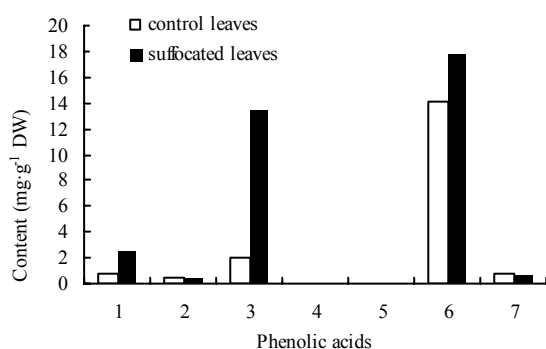


Fig. 4 Phenolic acids change in *P. simonii* × *P. pyramidalis* c.v leaves suffocated by MeSA.

The leaves treated with ethanol were used as control. The number from 1 to 7 along the X-axis stands for gallic acid, catechinic acid, pyrocatechol, caffeic acid, coumaric acid, ferulic acid and benzoic acid, respectively.

Discussion

Along with a constitutive resistance, plants possess mechanisms of active resistance preventing pathogen and herbivores invasion. There are many evidences suggest that phenolic acids may limit the host range of herbivore and prevent growth of competitive vegetation based on their activity as deterrents and

toxins (Harborne 1994). Increasing content of phenolic acids is considered as one measure of the activation of defense response. For example, flavonoids are larval growth inhibitors of the European corn borer (*Ostrinia nubilalis* Hubner) (Abou-Zaid *et al.* 1993). In this experiment, the change pattern of phenolic acids is more complex. The tendency of coumaric acid, caffeic acid and ferulic acid in *Populus deltoides* leaves exposed to MeJA is similar to that in *Populus deltoides* leaves exposed to MeSA; but there was a different phenolic acid content in poplar opera leaves and *Populus deltoides* leaves treated with MeJA or MeSA. This indicated that different species of plants had probably evolved differing levels of defense responses. Coumaric acid, caffeic acid and ferulic acid are central intermediates of lignin biosynthesis, and derivate from phenylalanine by the action of PAL (Dixon *et al.* 1995). Lignin accumulation can prevent xylem invasion due to an increase in the cell-wall mechanical rigidity (Stafford 1988).

Pyrocatechol, an orthodihydroxy phenol, may be oxidated by peroxidase (POD) and polyphenol oxidase (PPO) to corresponding quinones in response to herbivores attack and pathogen invasion (Bajaj 1998). In our experiment, the content of pyrocatechol stably increases in two poplars. Li Hui-ping (2003) suggested that the contents of phenolic acids were positively related to the resistance of poplar, and the resistance of poplar increased with contents of phenolic acids. Biosynthesis and accumulation of benzoic acid had been reported as a phytoalexin against fungal pathogen (Harborne 1994). Because benzoic acid was the precursor of salicylic acid (SA), it may serve as supply to induce accumulation of SA in damaged plants. SA has been shown to be signal for systemic acquired resistance of plants (Mauch-Mani *et al.* 1996). In our experiment, benzoic acid increased in *Populus deltoides* leaves treated with MeJA and MeSA, but decreased in poplar opera leaves treated with MeJA and MeSA. The reason may be that benzoic acid in poplar opera leaves exposed to MeJA and MeSA was used to synthesize salicylic acid to defense the herbivore and pathogen attack.

Gallic acid is typical hydrolysable tannin, and catechinic acid is the building block of condensed tannins (Higuchi 1997). Tannins reduce the edibility of plant tissue and inhibit the digestion of protein in herbivores. Tannins, as well as lignin, also increase the leaf toughness (Lambers *et al.* 1998). Gallic acid content in *Populus deltoides* leaves treated with MeJA comes to a peak at 24 h while to a peak at 12-d having leaves treated with MeSA. Catechinic acid has no significant change in *Populus deltoides* leaves treated with MeSA and MeJA. It indicated that different patterns of phenolic acids may manifest different resistance in response to attack on plant. These results supported that MeJA and MeSA could function as airborne signals inducing resistance of poplars. One possible reason of the discrepancy between previous studies and ours may be the material we chose, since most other studies have focus on model plants such as tobacco (Karban *et al.* 2000) and tomato (Farmer *et al.* 1990). This indicates that communication between plants can occur and is mediated by an airborne signal.

In our opinion, if the resistance to pathogen and herbivore is successful development, plants should offer active basic metabolism and active phenolic acids metabolism, remain viable after invasion. Our study on the defensive responses of poplar has revealed differences in the contents of phenolic acids in plants exposed to volatile compounds. It concluded that MeSA and MeJA might act as airborne signal molecules to induce an un-wound neighboring plant to enhance resistance. However,

whether such signal molecules are universal in nature or how these signal molecules can be identified and transported between plants need to make a further research.

References

- Abou-Zaid, M.M., Beninger, C.W., Arnason, J.T., Nozzolillo, C. 1993. The effect of one flavanone, two catechins and four flavonols on mortality and growth of the European corn borer (*Ostrinia nubilalis* Hubner) [J]. *Biochem. Syst. Ecol.*, **21**: 415–420.
- Bajaj, K.L. 1998. Biochemical basis for disease resistance-role of plant phenolics [C]. In: Singh, R., Sawhney, S.K. (eds), *Advances in frontier areas of plant biochemistry*. New Delhi: Prentice-Hall of India Private Limited, p487–510.
- Baldwin, I.T., Schultz, J.C. 1983. Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants [J]. *Science*, **221**: 277–278.
- Dempsey, D.M.A., Shah, J., Klessig, D.F. 1999. Salicylic acid and disease resistance in plants [J]. *Crit. Rev. Plant. Sci.*, **18**: 547–575.
- Dicke, M. 1999. Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? [J]. *Entomol. Exp. Appl.*, **91**: 131–142.
- Dick, M., Bruin, J. 2001. Chemical information transfer between damaged and undamaged plants [J]. *Biochem. Syst. Ecol.*, **29**: 979–980.
- Dixon, R.A., Paiva, N.L. 1995. Stress-induced phenylpropanoid metabolism [J]. *The Plant Cell*, **7**: 1085–1097.
- Farmer, E.E., Ryan, C.A. 1990. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves [J]. *Proc. Natl. Acad. Sci.*, **87**: 7713–7716.
- Farmer, E.E., Weber, H., Bollenweider, S. 1998. Fatty acid signalling in *Arabidopsis* [J]. *Planta*, **206**: 167–174.
- Harborne, J.B. 1994. *Introduction to Ecological Biochemistry*, 4th edn. [M]. New York: Academic Press.
- Higuchi, T. 1997. *Biochemistry and molecular biology of wood* [M]. Berlin Heidelberg New York: Springer-Verlag, p131–236.
- Karban, R., Baldwin, I.T., Baxter, K.J., Laue, G., Felton, G.W. 2000. Communication between plants: induced resistance in wild tobacco plants following clipping of neighboring sagebrush [J]. *Oecologia*, **125**: 66–71.
- Lambers, H., Chapin III, F.S., Pons, T.L. 1998. *Plant Physiological Ecology* [M]. New York: Springer-Verlag, p413–436.
- Li Huiping, Wang Zhigang, Yang Minsheng, *et al.* 2003. The relation between tannin and phenol constituents and resistance to *Anoplophora glabripennis* of various poplar tree species [J]. *Journal of Agricultural University of Hebei*, **26**(1): 36–39. (In Chinese)
- Mauch-Mani, B., Slusrenko. 1996. Production of salicylic acid precursors is a major function of phenylalanine ammonia-lyase in the resistance of *Arabidopsis* to *peronospora parasitica* [J]. *The Plant Cell*, **8**: 203–212.
- Mieczyslaw, K., Marzanna, S.W., Jeffrey, M.L., Alicja, M.Z. 1999. Cytochemical localization of phenolic compounds in columella cells of the root cap in seeds of *Brassica napus*-changes in the localization of phenolic compounds during germination [J]. *Annals of Botany*, **84**: 135–143.
- McConn, M., Creelman, R.A., Bell, E., Mullet, J.E., Browse, J. 1997. Jasmonate is essential for insect defense in *Arabidopsis* [J]. *Proc. Natl. Acad. Sci.*, **94**: 5473–5477.
- Paré, P.W., Tumlinson, J.H. 1999. Plant volatiles as a defense against insect herbivores [J]. *Plant Physiol.*, **121**: 325–331.
- Ryan, C.A. 2000. The systemin signaling pathway: differential activation of defensive genes [J]. *Biochem. Biophys. Acta.*, **1477**: 112–122.
- Shah, J., Klessig, D.F. 1999. Salicylic acid: Signal perception and transduction [C]. In: Libbenga, K., Hall, M., Hooykaas, P.J. (eds), *Biochemistry and Molecular Biology of Plant. Hormones Oxford: Elsevier*, p513–541.
- Shulaev, V., Silverman, P., Raskin, I. 1997. Airborne signalling by methyl salicylate in plant pathogen resistance [J]. *Nature*, **385**: 718–721.
- Stafford, N.A. 1988. Proanthocyanidins and the lignin connection [J]. *Phytochemistry*, **27**: 1–6.
- Wagner, M.R. 1988. Induced defenses in ponderosa pine against defoliating insects [C]. In: Mattson, W.J., Levieux, J., Bernard-Dagan, C. (eds), *Mechanism of woody plant defenses against insects*. New York: Springer-Verlag, p141–155.